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TITLE: Development of Artificial Antigen Presenting Cells for Prostate Cancer Immunotherapy

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13. Abstract *(Maximum 200 Words)* *(abstract should contain no proprietary or confidential information)*

A major goal in cancer immunotherapy is to generate an effective anti-tumor immune response. Adoptive immunotherapy involves stimulation of tumor-specific T cells, *ex vivo* (outside the body), followed by transfer of expanded numbers of activated T cells back into patients. While adoptive immunotherapy holds promise as a treatment for cancer, development of adoptive immunotherapy has been impeded by the lack of a reproducible and economically viable method for generating therapeutic numbers of antigen-specific CTL. The work proposed in this application will enable advances in adoptive immunotherapy.

Most prostate cancers express prostate specific molecules. These molecules, including PSA and PMSA, can serve as potential targets for immune-based treatments. Studies on immune recognition of these molecules have already identified potential target regions within these proteins and are the basis of a variety of different experimental immunotherapies for treatment of prostate cancer. In this study we propose to study the ability to use HLA-Ig based aAPC as a viable method for induction, expansion and activation of prostate specific T cells for immunotherapy for prostate cancer. These studies will serve as precursor ones for induction and expansion of prostate specific CTL from patients with disease for initiation of adoptive immunotherapy phase I clinical studies.

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Introduction:

Over the last year our focus has been on development of an animal model, as described in the original Specific Aim #2, to test the in vivo efficacy of aAPC induced prostate cancer specific CTL. This has permitted us to advance our studies while we simultaneously obtained approval from both the JHMI IRB and DOD for our human CTL expansion studies (see appendix A and B). We now have the components in place to work on Specific Aim #1 induction of anti-PSA-3A and PSA-1 prostate-specific CTL and to test their in vivo efficacy as described in Specific Aim #2.

Background: An Overview of Adoptive Immunotherapy

A major goal in the field of immunotherapy is to generate an effective cell-mediated anti-tumor immune response. Adoptive immunotherapy involves induction and expansion of antigen-specific T cells, *ex vivo*, followed by transfer of autologous antigen-specific T cells back into patients.

It has been demonstrated in both animal and human models that *ex vivo* expanded MHC Class I-restricted CD8+ cytotoxic T-lymphocytes (CTL) are able to kill virus-infected cells and tumor cells. While antigen-specific, antiviral CTL were first evaluated in humans by investigators at the Fred Hutchinson Cancer Research Institute [1, 2] who administered *ex vivo* expanded cytomegalovirus (CMV)-specific CTL clones as prophylaxis for CMV disease, Rosenberg's group [3] at the National Cancer Institute was first to successfully expand autologous tumor-specific T cells, referred to as tumor-infiltrating lymphocytes (TIL), *ex vivo*, and re-infuse them into melanoma patients together with IL-2 to help maintain both numbers and function of the re-infused cells. In a modest percentage of responding patients, they were able to show that re-infused TIL had trafficked back to tumor sites and directly induced tumor shrinkage, *in vivo*. Recent work has shown that adoptive transfer, in patients with metastatic melanoma, following a non-myeloablative conditioning regimen resulted in regression of the patients' metastatic melanoma as well as the onset of autoimmune melanocyte destruction [4]. More recent studies also show that adoptively transferred CTL can survive in patients with metastatic melanoma and track to the site of the tumor [5]. All these studies highlight the importance of exploring adoptive immunotherapy as a promising means to generate an effective cell-mediated anti-tumor/viral immune response for expanded clinical evaluation.

The development of an artificial Antigen-Presenting Cell (aAPC) has opened the gateway for *ex vivo* stimulation and expansion of tumor-specific T cells to clinically relevant numbers. Initially, June and colleagues developed approaches for non-specific expansion of CTL derived either from TIL-cultures or tetramer-based sorting for enrichment of antigen-specific CTL. By coupling beads to anti-CD3 and anti-CD28 mAbs, they have been able to expand CD4+ T cells. However, this non-specific system is limited in two ways. First, such anti-CD3/anti-CD28 beads failed to support long-term growth of CD8+ CTL, even when T cell growth factor (e.g. IL-2) was added. Second, it was not possible to maintain the antigen-specificity during expansion [6, 7], both

significant requirements for induction and expansion of tumor-specific T cells to clinically relevant quantities. Our preliminary data demonstrate that an artificial Antigen Presenting Cell (aAPC), made by coupling HLA A2-Ig and anti-CD28 to beads, can reliably induce and expand antigen-specific CTL from healthy donors. This approach promises to be a facile, cost-effective, and excellent alternative to the more time consuming and expensive dendritic cell based expansion. In our preliminary data, which supported funding of the grant, we showed that this approach can be used to expand multiple clinically relevant CTL populations including CTL specific for CMV or for melanoma antigens. In the current application we proposed to demonstrate functional efficacy of HLA-Ig complexes conjugated to beads as artificial Antigen Presenting Cells (aAPC) for inducing and expanding anti-PSA-3A and PSA-1 prostate-specific CTL. The specific aims envisioned are to *1) optimize aAPC structure and duration of stimulation, and 2) analyze the in vivo function of aAPC-induced CTL.*

Body:

Over the last year we focussed on development of a in vivo model system to test the efficacy of HLA-Ig-based aAPC induced prostate-specific CTL, as detailed in Specific Aim #2. To accomplish this, we developed a human/SCID mouse model of prostate cancer in our lab. While the human/SCID models [8-10] are by their nature only partially reconstituted immune responses, several of these models have been shown to have efficacy in studying adoptive immunotherapeutic treatment approaches for diseases including but not limited to EBV-associated lymphoma, adenocarcinoma and melanoma [8, 11-13]. In these models, adoptive immunotherapy has led to regression of transplanted tumors. Therefore the human/SCID model was chosen for our experiments.

We have developed two model systems that both show growth of human prostate cancer tumors in immunocompromised/SCID mice. Our experiments indicate (see Figure 10) that injection of 3.0×10^6 LNCaP (Figure 10A) results in reliable tumor growth in SCID/beige mice. In these animal tumors were first palpable at day 10 after injection of the LNCaP cells and grew to approximately 1.2 cm by day 35 when the mice were euthanized. In regular SCID mice tumor growth was also seen although it was delayed significantly. We also established the model using the human prostate cancer cells line PC-3 Figure 10B. Using this cell line tumor growth was more rapid with 0.8 cm tumors seen by day 7 that grew to approximately 1.3 cm during the course of the experiment. The PC-3 cell line used has also been used for in vivo visualization of tumor growth using bioluminescence of retrovirally transfected cells expressing a luciferase gene. Thus this approach can be used in our future experiments to examine the influence of adoptively transferred CTL on tumor growth in real time.

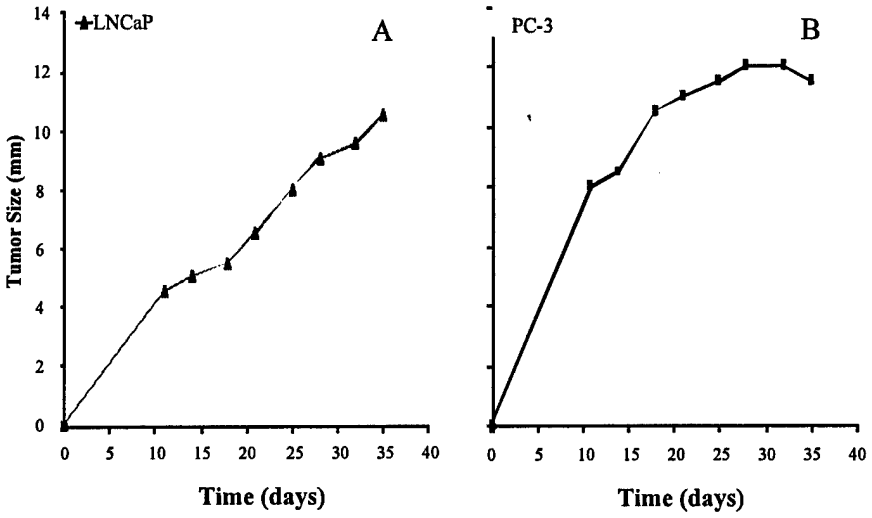

Figure Legend: SCID/beige mice received a subcutaneous injection of $3x10^6$ human prostate cancer cells LNCaP or PC3. The mice tumor size was measured on the indicated days using a caliper. The data set represents an average of 3 mice per group.

In this coming year we plan to use this model and inject PSA-1 or PSA3A-specific CTL (approximately 10^6) will be injected i.v. at days 3, 5, and 12 after tumor injection. Control animals will receive injections of PBS or irrelevant, CMV antigen-specific CTL. Our initial experiments will have: 1) a control group of 5 mice that receive tumor alone; 2) a treated group of 5 mice that in addition to getting the tumor cells will receive antigen-specific CTL; and 3) a group of 5 mice that in addition to getting the tumor cells will receive control noncognate CTL. This basic protocol will be done with both PSA-1 and PSA-3A specific CTL.

In addition we have obtained the reagents/cell lines required for development of various formulations of aAPC described in Specific Aim #1. These include B7.1-Ig B7.2-Ig, B7DC-Ig, and antibodies to 41-BB.

Bulleted List of Key Accomplishments:

1) Development of two model in vivo prostate tumor growth in SCID mice
    a. Using LNCaP cells
    b. PC-3
2) Obtained IRB approvals
3) Obtained material for starting work with human blood products including various different accessory molecules detailed in Specific Aim #1.

Reportable Outcomes:

A patent application is being planned but not yet submitted.

Summary:

To summarize, we have highlighted studies that demonstrate the importance of exploring adoptive immunotherapy as a logical method for treatment of prostate cancer. As discussed, the ability to produce clinically relevant amounts of antigen specific T-cells for adoptive transfer has been limited. Our method represents a novel approach, whereby the generation and expansion of antigen-specific CTL starting from low precursor frequency to significant amounts is facile and cost effective. Furthermore, the enhanced stimulatory capacity and specificity of the dimeric peptide-HLA-Ig constructs, when formulated with co-stimulatory molecules will provide a platform for a straightforward and reproducible cell culture process resulting in CTL activation and expansion useful for therapeutic applications.

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Date: Thu, 26 Aug 2004 16:22:55 -0400
From: "Duchesneau, Caryn L Ms USAMRMC" <caryn.duchesneau@us.army.mil>
Subject: A-11814: Approval Memo (Proposal No. PC020392,
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To: "Cardwell, Archie B Mr USAMRAA" <archie.cardwell@us.army.mil>
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Subject: Protocol, "Development of Artificial Antigen Presenting Cells for Prostate Cancer Immunotherapy," Submitted by Jonathan Schneck, MD, Johns Hopkins University School of Medicine, Baltimore, MD, Proposal No. PC020392, Award No. DAMD17-03-1-0370, HSRRB Log No. A-11814

1. Revisions to this protocol, made in response to the initial MFR, were received between May and August 2004. The protocol and supporting documents have been reviewed and have been found to comply with applicable human subjects protection regulations.

2. There are no outstanding humans subjects protection issues to be resolved. The study represents no greater than minimal risk and is eligible for expedited review under 32 CFR 219.110. Specifically, this study involves taking blood samples from healthy volunteers, and is approved for the use of human subjects at Johns Hopkins University.

3. The Use of Human Subjects Clause and the Use of Anatomical Substances Clause should be entered into the Assistance Agreement for work performed at this site.

4. A continuing review report was approved by the local IRB on 13 April 2004 stating that 41 subjects have been enrolled. However, these subjects are part of a larger study funded by NIH and are not supported by DOD funds.

5. Any protocol modifications (including, but not limited to, changes in the principal investigator, inclusion/exclusion criteria, number of subjects to be enrolled, study sites, or procedures) must be submitted as a written amendment for HSRRB review and approval before implementing the change. Documentation that the local IRB reviewed and approved the modifications must be submitted also.

6. In accordance with 32 CFR 219, a copy of the continuing review report approved by the IRB of Record should be submitted to this office as soon as possible after receipt of approval. It appears that the next continuing review report is due to the JCCI no later than 13 July 2005.

7. The Volunteer Registry Data Sheet is not required for this study.

8. The point of contact for this action is Donna S. Ferrandino, PhD at ext. 3-6237.

CARYN L. DUCHESNEAU, CIP
Vice Acting Chair, Human Subjects
Research Review Board

Note: The official signed copy of this approval is housed with the protocol file at the Office of Regulatory Compliance and Quality, 504 Scott Street, Fort Detrick, MD, 21702. Signed copies will be provided upon request.

JOHNS HOPKINS
MEDICINE

Institutional Review Boards

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JHM-IRB 3
AMENDMENT EXPEDITED APPROVAL NOTICE WITH REVISED CONSENT FORM

TO: Jonathan Schneck, MD/PhD
Professor, Pathology & Medicine
Ross 664G
School of Medicine

FROM: Paul S. Lietman, M.D., Ph.D.
Chairman - JHM-IRB 3

DATE: **August 25, 2004**

RE: **Application NO: 04-07-07-01, entitled, Development of Artificial Antigen Presenting Cells for Prostate Cancer Immunotherapy**
Amendments:
1. ***Consent form modified to include a place to record the permanent addresses of the subjects.***
2. ***Consent form modified to state that collected samples to be destroyed at the end of the study.***

1. The Committee received your letter of **08/02/2004**, requesting an amendment to the above-referenced protocol and consent form. In the Committee's opinion, the amendment qualified for an expedited review. There were no questions raised and approval was granted on **08/10/2004**. The attached stamped consent form, approved with the amendment, **listed above**, is valid from **08/10/2004** to **07/13/2005**.

2. No additional changes, amendments, or addenda may be made in this protocol without the Committee's review and approval.

3. The action taken on this protocol does not change the annual renewal date, which remains **07/13/2005**.

PL:sjs

Enclosures